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Research paper

THP-1 and human peripheral blood mononuclear cell-derived macrophages differ in their capacity to polarize in vitro

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ABSTRACT

Macrophages (Mφ) undergo activation to pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes in response to pathophysiologic stimuli and dysregulation of the M1-M2 balance is often associated with diseases. Therefore, studying mechanisms of macrophage polarization may reveal new drug targets. Human Mφ polarization is generally studied in primary monocyte-derived Mφ (PBMC Mφ) and THP-1-derived Mφ (THP-1 Mφ). We compared the polarization profile of THP-1 Mφ with that of PBMC Mφ to assess the alternative use of THP-1 for polarization studies. Cellular morphology, the expression profiles of 18 genes and 4 cell surface proteins, and phagocytosis capacity for apoptotic cells and *S. aureus* bioparticles were compared between these Mφ, activated towards M1, M2a, or M2c subsets by stimulation with LPS/IFNγ, IL-4, or IL-10, respectively, for 6 h, 24 h and 48 h. The Mφ types are unique in morphology and basal expression of polarization marker genes, particularly CCL22, in a pre-polarized state, and were differentially sensitive to polarization stimuli. Generally, M1 markers were instantly induced and gradually decreased, while M2 markers were markedly expressed at a later time. Expression profiles of M1 markers were similar between the polarized Mφ types, but M2a cell surface markers demonstrated an IL-4-dependent upregulation only in PBMC Mφ. Polarized THP-1 Mφ but not PBMC Mφ showed distinctive phagocytic capacity for apoptotic cells and bacterial antigens, respectively. In conclusion, our data suggest that THP-1 may be useful for performing studies involving phagocytosis and M1 polarization, rather than M2 polarization.

1. Introduction

Macrophages (Mφ) are antigen-presenting cells that distribute to peripheral tissues where they play multiple roles in diverse physiological processes including host defense, inflammation resolution and tissue remodeling (Mosser and Edwards, 2008; Gilroy and De Maeyer, 2015). Mφ are highly plastic and activated into different phenotypic subsets with distinct properties: two extremes being pro-inflammatory (M1) and anti-inflammatory (M2), depending on the ambient stimulus and anatomical location (Gordon, 2007). During tissue damage or pathogen exposure, Mφ are activated toward the M1 subset by Th1 cytokines such as IFNγ and TNFα in combination with Toll-like receptor ligands like lipopolysaccharide (LPS), and produce pro-inflammatory cytokines to kill intracellular microorganisms and induce Th1 immunity. In contrast, the M2 subtype exerts a wide range of functions including immune regulation mediated by the production of anti-inflammatory cytokines, scavenging of apoptotic cells and debris and tissue remodeling. M2 Mφ are further classified into different groups;

Abbreviations: ACTB, β-actin; CCL2, chemokine (C-C motif) ligand 2; CCL18, chemokine (C-C motif) ligand 18; CCL22, chemokine (C-C motif) ligand 22; CCR7, chemokine (C-C motif) receptor 7; CD, cluster of differentiation; COX2, cytochrome C oxidase-2; IFNγ, interferon-γ; IL1β, interleukin 1β; IL-4, interleukin-4; IL-10, interleukin 10; IRF4, interferon regulatory factor 4; IRF5, interferon regulatory factor 5; Jmjd3, JmjC domain-containing protein 3; LPS, lipopolysaccharide; Mφ, macrophages; MRC1, mannose receptor C type 1; PBS, phosphate buffered saline; PMA, phorbol 12-myristate 13-acetate; PPARγ, peroxisome proliferator-activated receptor γ; PBMC Mφ, primary monocyte-derived Mφ; RelA, nuclear factor kappa B p65; RelB, nuclear factor kappa B; RPL37A, ribosomal protein L37a; SOCS1, suppressor of cytokine signalling 1; SOCS3, suppressor of cytokine signalling 3; SSP, staurosporine; TGFβ1, transforming growth factor β; THP-1 MΦ, THP-1-derived Mφ; TLP, toll-like-receptor; TNFα, tumour necrosis factor α

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M2a, M2b and M2c, based on the polarization stimulus resulting in a different expression profile of marker genes (Murray et al., 2014; Muraille et al., 2014; Lawrence and Natoli, 2011; Wang et al., 2014; Motwani and Gilroy, 2015; Spiller et al., 2016). M2a and M2c phenotypes, induced by either the Th2 cytokines IL-4/IL-13 or the anti-inflammatory IL-10, respectively, have been investigated most extensively among M2 subsets to gain an understanding of the roles of Mφ in immune regulation and inflammation resolution (Murray et al., 2014; Spiller et al., 2016; Martinez and Gordon, 2014; Zizzo and Cohen, 2015). Dysregulated Mφ polarization is often associated with disease onset and progression in various diseases including chronic inflammatory diseases and cancers (Mosser and Edwards, 2008; Neele et al., 2015; Murray and Wynn, 2011). Consequently, Mφ subsets are considered as potential therapeutic targets due to their high plasticity, their influence on the surrounding microenvironment via cytokine/chemokine production and their relevance to disease progression.

A number of Mφ polarization markers have been proposed in both mouse and human models, but these markers are far from being consistent among each other (Murray et al., 2014; Muraille et al., 2014; Wang et al., 2014; Martinez and Gordon, 2014; Vogel et al., 2014). Human Mφ polarization is frequently studied using in vitro differentiated Mφ prepared from peripheral blood mononuclear cells (PBMC) or monocytic cell lines (Spiller et al., 2016; Vogel et al., 2014; Tedesco et al., 2015; Chanput et al., 2014), because primary tissue macrophages cannot be readily expanded *ex vivo* and their isolation is invasive and labour-intensive. The isolation of monocytes from peripheral blood is laborious and their subsequent differentiation to PBMC-derived macrophages (PBMC Mφ) lasts over 6 days in standard protocols (Tedesco et al., 2015; Namgaladze et al., 2015; Pena et al., 2011; Mia et al., 2014), whereas cell line-derived Mφ can be prepared within a much shorter period (Spiller et al., 2016; Spencer et al., 2010; Chanput et al., 2013). There are notable advantages in the use of cell line-derived Mφ over PBMC Mφ: (i) easy acquisition and handling, (ii) unlimited cell number, (iii) homogeneous genetic/epigenetic backgrounds and (iv) purity of Mφ population (Chanput et al., 2014; Qin, 2012). However, cell culture conditions (medium and supplements, cell culture plate type and culture duration) during in vitro differentiation influence the resulting Mφ phenotypes (Murray et al., 2014) creating difficulties when comparing results from different cell phenotypes. In vitro differentiated macrophage-like cells, obtained by exposure of human monocytic cell lines to phorbol 12-myristate 13-acetate (PMA), on the other hand, have been widely used to study Mφ biology (Chanput et al., 2014; Chanput et al., 2013; Daigneault et al., 2010; Vance et al., 2016; Song et al., 2015; Mengubas et al., 1996). It has been shown that THP-1 derived macrophage-like cells (THP-1 Mφ) resemble primary Mφ in some functional properties and differentiation markers (Chanput et al., 2014; Daigneault et al., 2010; Chen et al., 2016a; Pedraza-Brindis et al., 2016; Sharif et al., 2007). Moreover, the differentiation and polarization phenotypes of THP-1 Mφ in comparison with PBMC Mφ have been analysed to some extent, for instance, phagocytic function and apoptosis resistance in a pre-polarized state, and expression profile of polarization marker genes in stimulated Mφ (Spiller et al., 2016; Daigneault et al., 2010; Sharif et al., 2007). However, neither the expression profile of polarization markers over a broad time range nor the phagocytic capacity of polarized Mφ have been compared.

In the current study, we evaluated surface and signaling markers for the polarization of THP-1 Mφ in comparison to those in primary monocyte-derived Mφ to assess the alternative use of THP-1 Mφ for polarization studies. The morphology and basal expression level of 18 putative polarization marker genes were investigated in both pre-polarized Mφ cell types . We then analysed the expression profile of the 18 marker genes and four cell surface marker proteins in M1, M2a and M2c subsets for each model at 6 h, 24 h and 48 h after stimulation. Furthermore, functional differences in phagocytosis of apoptotic cells and bacterial particles were assessed in these polarized Mφ types.

2. Materials and methods

2.1. Compounds

We prepared 1 mg/ml stock solutions of phorbol 12-myristate 13-acetate (PMA) and staurosporine (SSP), both from Sigma-Aldrich, St. Louis, MO, USA, and a 5 mM stock solution of Cell Proliferation Dye Fluor® 670 (eBioscience, San Diego, CA, USA) and cytochalasin D (Sigma) in cell culture-grade dimethyl sulfoxide (DMSO) from Carl Roth (Karlsruhe, Germany). Stock solutions (10 μg/ml) of recombinant human interferon-γ (IFNγ) from Thermo Fisher Scientific (Oberhausen, Germany), *Escherichia coli* lipopolysaccharide (LPS), interleukin-4 (IL-4) and interleukin-10 (IL-10), all from Sigma-Aldrich, were prepared in cell culture-grade phosphate buffered saline (PBS). All stock solutions were stored at −20° C and the desired concentrations of compounds were freshly prepared in cell culture medium for each treatment.

2.2. In vitro differentiation of THP-1 cells

Human monocytic cell line THP-1 was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and maintained in RPMI 1640 GlutaMAX™ medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and a 1% (v/v) mixture of penicillin and streptomycin, all reagents from Thermo Fisher Scientific, in a humidified incubator containing 5% CO_2 at 37° C. Periodically, the cells were controlled for mycoplasma contamination using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, Basel, Switzerland). In order to obtain THP-1 Mφ, THP-1 cells were seeded at a density of 0.5×10^6 cells per well in a six-well culture plate and stimulated with 50 ng/ml of PMA for 48 h, followed by further incubation in complete medium in the absence of PMA for 3 d.

2.3. On-plate monocyte selection and macrophage differentiation

Peripheral blood mononuclear cells (PBMCs) were isolated from blood buffy coats (DRK-Blutspendedienst Baden-Wuerttemberg-Hessen, Germany) using a Histopaque® 1077 density gradient (Sigma-Aldrich). The PBMCs were washed twice with PBS containing 2 mM EDTA (PBS-EDTA) and incubated in an erythrocyte lysis buffer (135 mM NH_4Cl, 10 mM $NaHCO_3$, 0.1 mM Na-EDTA, pH.7.2) for 3 min at room temperature. Subsequently, PBMCs suspended in serum-free RPMI 1640 medium supplemented with 1% (v/v) gentamycin (Sigma-Aldrich) were seeded at a density of $1–1.5 \times 10^7$ cells per well in a six-well culture plate for 1 h in a humidified incubator containing 5% CO_2 at 37 °C to allow monocyte adhesion. Non-adherent cells were removed and the adherent monocytes were further incubated in RPMI 1640 medium supplemented with 2.5% (v/v) heat-inactivated human serum and 1% (v/v) gentamycin for 7 d with PBS-EDTA wash and media replacement every 3 d to obtain matured macrophages (PBMC Mφ). Written informed consent that allows blood for research use was provided by the serum donor.

2.4. In vitro polarization of macrophages

For M1 polarization, Mφ in fresh medium were co-stimulated with LPS (50 ng/ml) and IFNγ (20 mg/ml) whereas the M2a and M2c subsets were obtained by stimulation with either IL-4 (20 mg/ml) or IL-10 (20 mg/ml), respectively. Mφ were incubated with the corresponding stimuli for 6, 24 and 48 h without media replacement.

2.5. Phase-contrast microscopy

The THP-1 Mφ and PBMC Mφ were washed once with PBS and the morphology of adherent cells was photographed using an Observer.Z1. (Carl Zeiss, Oberkochen, Germany) equipped with AxioCam MRC